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Bark-Inhabiting Green Algae in Japan (3) *Chlorella trebouxioides* and *Ch. angusto ellipsoidea,* sp. nov, (Chlorelloideae, Chlorellaceae, Chlorococcales)

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Two species of bark-inhabiting *Chlorella* were collected from the Uchibo area, Chiba Prefecture, Japan. One is ovoid in shape and the other is elongately ellipsoidal. The chloroplast of these two algae is basically identical and band-shaped with irregularly undulated margin. The pyrenoid structure is also almost the same in both algae. The pyrenoid is penetrated by thylakoids which irregularly undulate or encircle, and surrounded by many small starch grains. Pyrenoglobuli are found at the periphery of the pyrenoid and rarely around thylakoids. The autospores produced in the ovoid alga are unequal in size, while those of the elongately ellipsoidal alga are almost equal. The ovoid alga is identified as *Ch. trebouxioides* Punčochářová. The elongate alga is recognized as a new species *Chlorella angusto ellipsoidea* Hanagata & Chihara.

(Continued from J. Jpn. Bot. **71**: 310–316, 1996)

Introduction

In the course of our studies of bark-inhabiting green algae, many specimens of *Chlorella* and the related algae were collected from the Uchibo area, Chiba Prefecture, Japan. We have carried out the taxonomic studies of these algae with light and electron microscopy. *Scenedesmus komarekii* Hegewald, *Coelastrella mutistriata* Kalina & Punčochářová var. *multistriata, Chlorella ellipsoidea* Gerneck and *Ch. luteoviridis* Chodat were previously reported (Hanagata et al. 1996a, 1996b). In this paper, *Ch. trebouxioides* Punčochářová and a new species, *Ch. angusto ellipsoidea* Hanagata & Chihara, are considered.

Materials and Methods

Chlorella trebouxioides and *Ch. angusto-ellipsoidea* were collected from the bark of *Zelkova serrata* (Thunb.) Makino, on 5 June, 1993, at Goi, Ichihara City, and at Inage, Chiba City, respectively. The algae examined in this study were cultured in Bold's basal medium (1949). The methods of unialgal culture and procedure of transmission electron microscopy were described in a previous paper (Hanagata et al. 1996a).

Results and Discussion

Chlorella trebouxioides Punčochářová

(Figs. 1 and 2)

Light microscopy

The alga is unicellular and solitary. Young cells are ellipsoidal, ovoid or sometimes spherical to subspherical, 3.0–7.0 × 4.0–9.0 μm in size or 4.0–7 μm in diameter. Mature cells are spherical or ovoid to sometimes ellipsoidal, 6.0–17 μm in diameter or 5.0–10 × 7.0–13 μm in size. Each cell has a single chloroplast. The chloroplast in young cells is parietal and band- or saucer-shaped with relatively smooth margin, while that in mature cells is irregularly undulated or slightly twisted, sometimes incised, and often separated from the cell wall here and there. The pyrenoid is conspicuous, spherical to subspherical, and surrounded by divided small starch grains. Oil droplets are numerous in cytoplasm and vacuoles are sometimes present in mature cells. Cells are capable of producing 2–16 autospores. Reproduction begins with cleavage of protoplast into an irregular number of portions unequal in size. In addition to the small autospores, there are one or several larger autospores. Small autospores are ellipsoidal to ovoid or spherical, 2.0–6.0 × 1.5–4.5 μm in size or 2.0–6.0 μm in diameter, while large ones ovoid or spherical, 6.0–9.0 × 4.5–6.5 μm in size or 6.0–10 μm

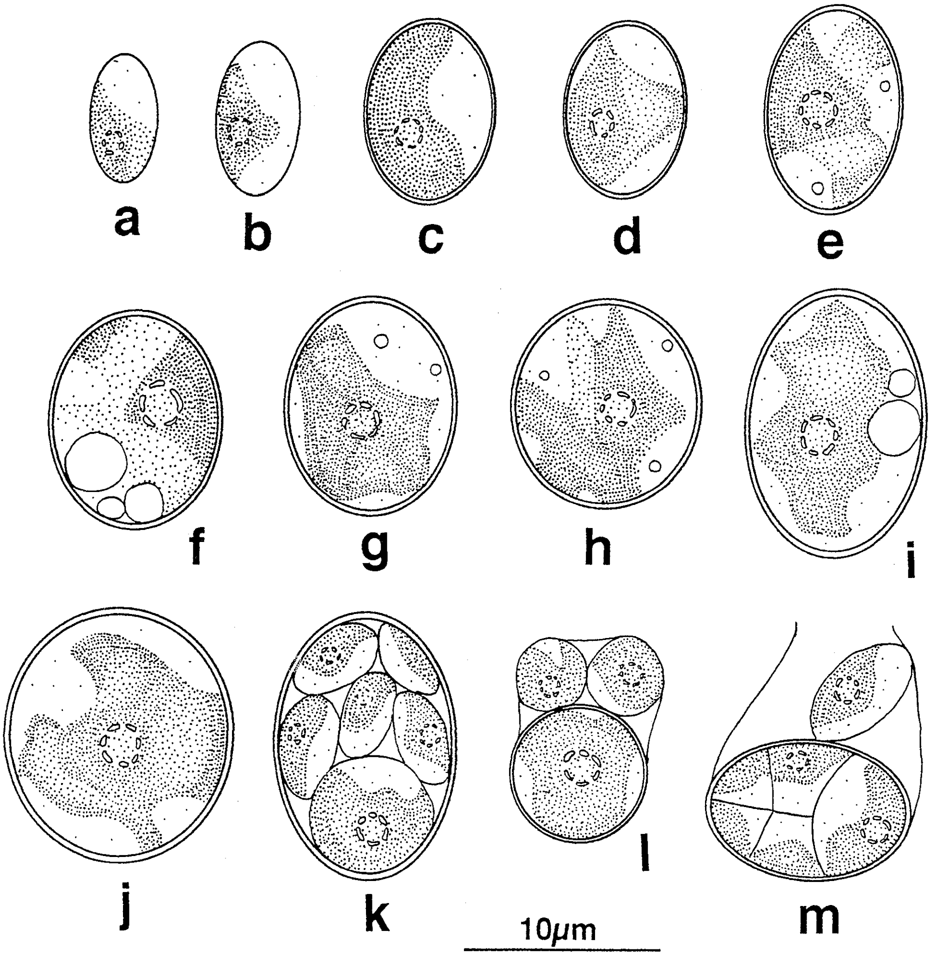

Fig. 1. *Chlorella trebouxioides*. a–d. Young vegetative cells. e–j. Mature vegetative cells. k, l. Autospores in parent cells. m. Ruptured parent cell.

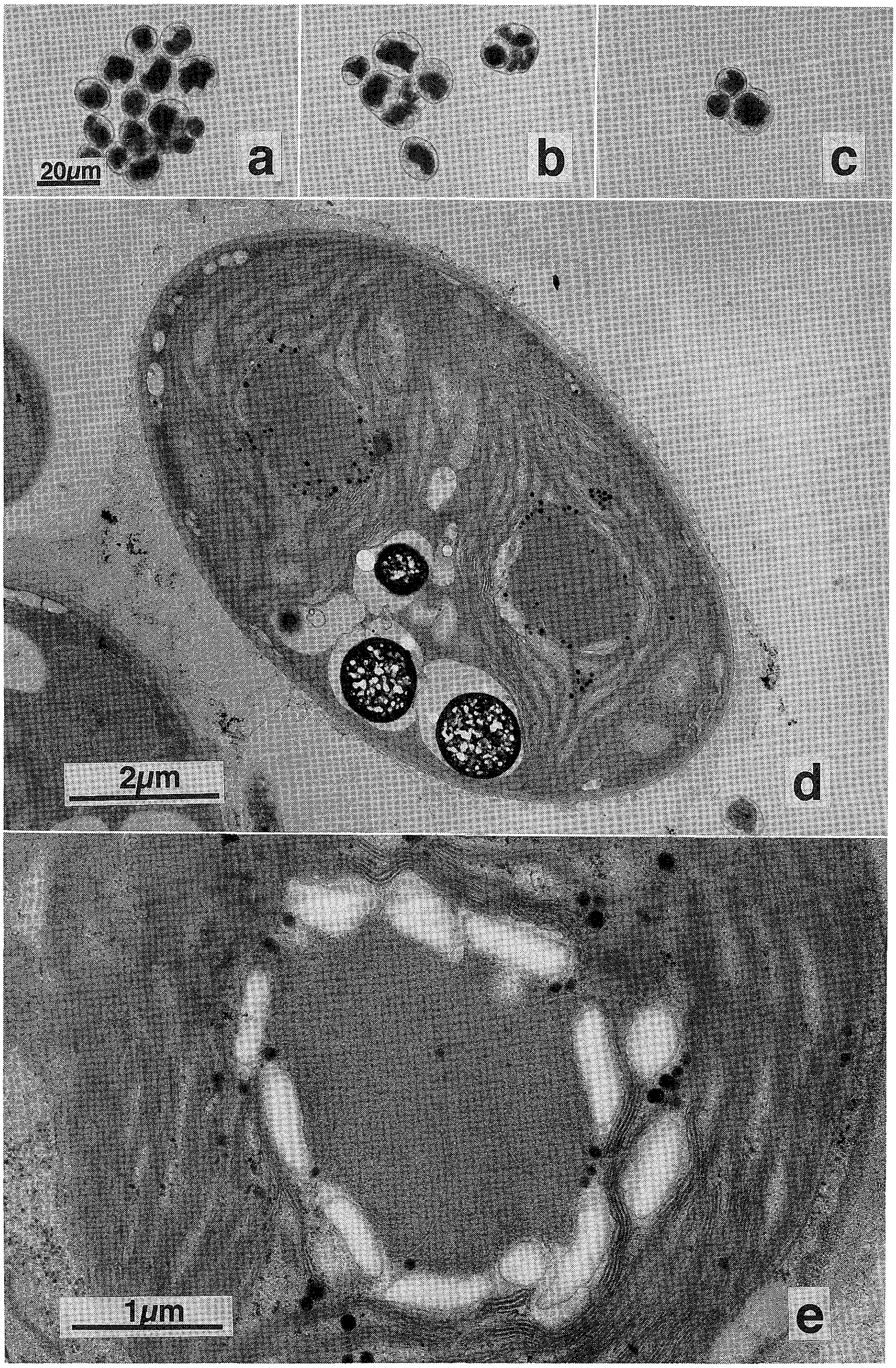

Fig. 2. *Chlorella trebouxioides*. (a–c. Photomicrographs. d, e. Transmission electron micrographs). a. Vegetative cells. b, c. Autospores. d. Section of vegetative cells. e. Section of pyrenoid, showing surrounded many starch grains and irregularly undulated thylakoids traversing the pyrenoid matrix.

in diameter. Large autospores often produce new autospores while still within the sporangium. The parent cell wall forms an aperture through which the autospores are discharged. Some autospores also can remain within the empty parent cell.

Electron microscopy

The cell wall is single in layer and 0.16–0.30 μm in thickness. The chloroplast consists of thylakoid lamellae arranged in several almost parallel rows. The thylakoid lamellae are in contact with the surface of the pyrenoid. Single thylakoids traverse the pyrenoid, and irregularly undulate in the pyrenoid (Fig. 2d, e). The pyrenoid is surrounded by numerous starch grains. Pyrenoglobuli are distributed at the periphery of the pyrenoid and rarely in the pyrenoid matrix. Plastglobuli are sometimes present among the thylakoids in the chloroplast.

The gross morphology of our alga resembles that of *Chlorella ellipsoidea* observed by Fott and Nováková (1969). They treated *Ch. ellipsoidea* as a variety of *Ch. saccharophila*. Many phycologists and biochemists examined *Ch. ellipsoidea* strain Pringsheim SAG 211-1a and CCAP 211/1a, and concluded that *Ch. ellipsoidea* should be an independent species rather than a variety of *Ch. saccharophila*. Recently, Punčochářová has pointed out that the observation of *Ch. ellipsoidea* by Fott and Nováková (1969) was not based upon these authentic strains, but upon the Prague Collection CAUP H1904. Skoda (1992) showed that the pigment composition of the strain CAUP H1904 differed from that of *Ch. ellipsoidea* SAG 211-1a. Punčochářová (1994) also introduced the personal communication with Takeda on the difference of cell wall composition in both taxa. She carefully studied the strain CAUP H1904, and determined it as a new species, *Ch. trebouxioides* Punčochářová (1994). The gross morphology of our alga is fundamentally identical with that of *Ch. trebouxioides*. The single thylakoids penetrate the pyrenoid matrix and irregularly undulate within the pyrenoid in our alga, a feature similar to that of *Ch. saccharophila* observed by Ikeda and Takeda (1995). Although the transmission electron micrograph was not shown, Punčochářová (1994) indicated that the pyrenoid of *Ch. trebouxioides* was penetrated by the twisted thylakoids.

***Chlorella angusto ellipsoidea* Hanagata & Chihara, sp. nov.** (Figs. 3 and 4)

Alga unicelluaris et solitaria. Cellulae juvenes anguste ellipsoideae, 2.5–5.0 × 4.0–12 μm. Cellulae maturae ellipsoideae vel aliquando late ellipsoideae, 3.5–8.0 × 6.0–15 μm. Chloroplastum cellulae juveniae parietale et vittiforme vel acetabuliforme. Chloroplastum cellulare maturae irregulariter vittiforme, marginem undulatum et incisum habens. Pyrenoides singula granis amyli numerosis circumcincta. Cellulae maturae vacuolas unas vel numerosas habentes. Matrix pyrenoidis per lamellam undulatam invasa. Reproductio asexualis per 4–16 autosporas magnitudinis aequalis effecta. Autosporae ellipsoideae vel anguste ellipsoideae, 2.5–3.5 × 4.0–10 μm, per aperturam parietis sporangii liberatae.

Holotype: Figures 3 and 4.

Type locality: Inage, Chiba City, Chiba Prefecture, Japan.

Habitat: Specimens examined were isolated from the samples attached on the bark of *Zelkova serrata* (Thunb.) Makino.

Type culture: Inage, Chiba City, Chiba Prefecture, 5 June 1993. Strain A7-4 is deposited in the Culture Collection at Chiba Laboratory, Mitsui Engineering & Shipbuilding Co., Ltd.

Light microscopy

The alga is unicellular and solitary. Young cells are elongately ellipsoidal, 2.5–5.0 × 4.0–12 μm in size. Mature cells are ellipsoidal and sometimes broadly ellipsoidal, 3.5–8.0 × 6.0–

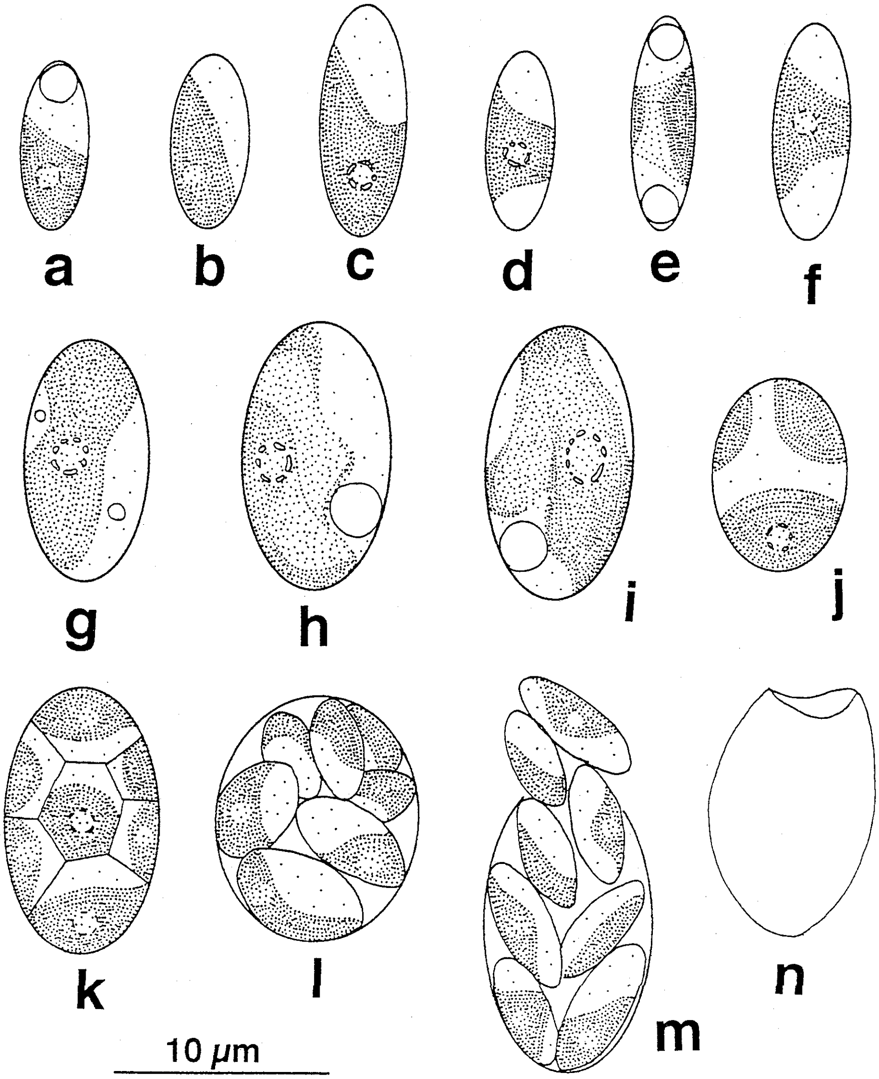

Fig. 3. *Chlorella angustoellipsoidea* sp. nov. a–f. Young vegetative cells. g–j. Mature vegetative cells. k, l. Autospores in parent cells. m. Ruptured parent cell. n. Empty parent cell wall.

15 μm in size. The chloroplast in young cells is parietal and band- or saucer-shaped with relatively smooth margin and that in mature cells is irregularly band-shaped with undulated margin and sometimes possesses incisions. The chloroplast has a pyrenoid that is sometimes inconspicuous in young cells, but distinct in mature cells. The pyrenoid in mature cells is covered with many starch grains. Vacuoles are one to numerous in mature cells. Cells are capable of producing 4–8–16 (rarely more than 16) autospores. Reproduction begins with cleavage of protoplast into an irregular number of almost equal portion in size. Autospores are narrowly ellipsoidal or elongately ellipsoidal, 2.5–3.5 × 4.0–10 μm in size. The parent cell wall dissolves at one place and forms an aperture through which the

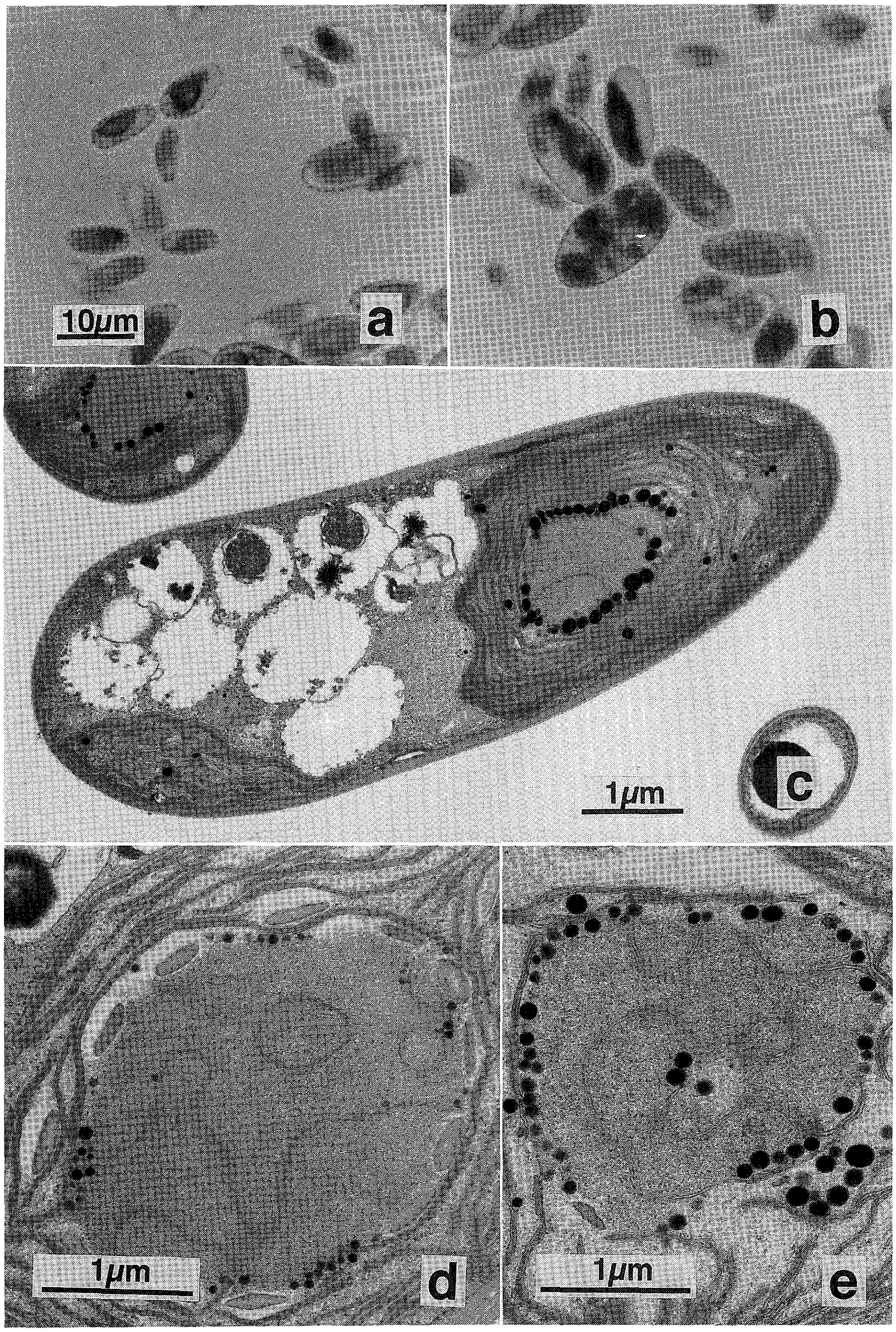

Fig. 4. *Chlorella angusto ellipsoidea* sp. nov. (a, b. Photomicrographs. c–e. Transmission electron micrographs). a. Young vegetative cells. b. Mature vegetative cells and autospores. c. Section of vegetative cells. d. Section of pyrenoid, showing many surrounding starch grains and irregularly undulated and encircling thylakoids penetrating the pyrenoid matrix. e. Localization of pyrenoglobuli in the pyrenoid.

autospores are discharged.

Electron microscopy

The cell wall is single in layer and 0.03–0.10 μm in thickness. The chloroplast consists of thylakoid lamellae aranged in several almost parallel rows. The pyrenoid matrix is spherical, 0.8–2.0 μm in diameter and located near the center of the chloroplast. The thylakoid lamellae are in contact with the surface of the pyrenoid. Single thylakoids branch off from the thylakoid lamellae and penetrate into the pyrenoid. These thylakoids irregularly undulate and encircle within the pyrenoid. Starch grains are located at the periphery of the pyrenoid and among the thylakoid lamellae. Pyrenoglobuli are always located at the periphery of the pyrenoid, and rarely distributed around the thylakoids in the pyrenoid. Plastoglobuli are often positioned around the thylakoids.

The new species is similar to *Pseudococcomyxa* or *Choricystis* in the shape of cells and chloroplast (Komárek and Fott, 1983), but differs from them by the presence of pyrenoid and the methods of autospore formation. These algae form 2–4 autospores, while our new species forms more than 4 autospores. The new species is also similar to E-type of cells designated for *Chlorella reisiglii* by Watanabe (1977) in the shape of cells and chloroplast at mature stage of growth. One of the specific features of *Ch. reisiglii* is the presence of two types of cells; one called the E-type is elongate-cylindrical or elongate-ellipsoidal and the other called the S-type is spherical or subspherical. In our new species, however, the S-type cells are not observed in any specimen from culture or from nature. Another feature to distinguish our new species from *Ch. reisiglii* is the shape of autospores and cells at young stage; in the latter the autospores and young cells are elongately cylindrical or curved-cylindrical, but in ours those are never cylindrical, and narrowly or elongately ellipsoidal.

The irregularly undulate thylakoid in the pyrenoid matrix of the new species is fundamentally the same as that of *Chlorella trebouxioides* (Punčochářová 1994) and *Ch. saccharophila* (Ikeda and Takeda 1995). The pyrenoglobuli in our alga always are positioned at the periphery of the pyrenoid matrix, and the localization does not change. A similar localization of pyrenoglobuli is also observed in the alga assigned to *Ch. trebouxioides* in this paper. The pyrenoid in our new species is surrounded by numerous small starch grains, a feature also found in *Ch. trebouxioides*. However, the new species differs from *Ch. trebouxioides* by the cell shape and the methods of autospore formation. *Ch. trebouxioides* forms autospores unequal in size and the ones remaining within the parent cell can produce more autospores, while our new species forms autospores equal in size and no autospores remain within the parent cell. *Ch. saccharophila* also differs from the new species by having no starch grains and pyrenoglobuli surrounded the pyrenoid as observed by Ikeda and Takeda (1995). We have been conducting the phylogenetic study using 18S rRNA coding gene sequences of this group of algae. The tree topologies obtained shows that our species is a sister group to *Chlorella saccharophila* (SAG 211-9a, the type strain), and hence these two taxa are considered to be closely related. We will present our data elsewhere.

For *Chlorella*-related algae the genus *Lobosphaera* (Reisigl 1964) and *Lobosphaeropsis* (Reisigl 1969) were established based on chloroplast feature (deep lobes with incisions). Apart from that, Tschermak-Woess (1980) founded the genus *Elliptochloris* for a coccoid green alga which produces two types of autospores; the spherical spores and the rod-like ones. With regard to the classification of the Chlorelloideae, however, we should hold off further discussion until molecular studies have been made.

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References

Bold H. 1949. The morphology of *Chlamydomonas chlamydogama* sp. nov.. Bull. Torrey Bot. Club **70**: 101–108.

Fott B. and Nováková M. 1969. A monograph of the genus *Chlorella*, the fresh water species. *In*: Fott B. (ed.), Studies in Phycology: 10–74. Stuttgart.

Hanagata N., Karube I. and Chihara M. 1996a. Bark-inhabiting green algae in Japan (1). *Scendesmus komarekii* and *Coelastrella multistriata* var. *multistriata* (Scotiellocystoideae, Chlorellaceae, Chlorophyceae). J. Jpn. Bot. **71**: 87–97.

———, ——— and ——— 1996b. Bark-inhabiting green algae in Japan (2). *Chlorella ellipsoidea* and *Ch. luteoviridis* (Chlorelloideae, Chlorellaceae, Chlorococcales). J. Jpn. Bot. **71**: 310–316.

Ikeda T. and Takeda H. 1995. Species-specific differences of pyrenoids in *Chlorella* (Chlorophyta). J. Phycol. **31**: 813–818.

Komárek J. and Fott B. 1983. Chlorophyceae (Grünalgen), Ordnung: Chlorococcales. *In*: Huber-prestalozzi G. (ed.), Das Phytoplankton des Süsswassers. Systematik und Biologie 7(1), 1044 pp. Stuttgart.

Punčochářová M. 1994. A taxonomic study of two *Chlorella* strains. Arch. Protistenkd. **144**: 237–247.

Reisigl H. 1964. Zur Systematik und Ökologie alpiner Bodenalgen. Österr. Bot. Z. **111**: 402–499.

——— 1969. Bodenalgen-Studien II. Österr. Bot. Z. **116**: 492–506.

Skoda B. 1992. Contributions to the biochemical taxonomy of the genus *Chlorella* Beijerinck sensu lato – Pigment composition. I. Cultures growing under optimal conditions. Algol. Studies. **63**: 19–35.

Tschermark-Woess E. 1980. *Elliptochloris bilobata*, gen. et spec. nov., der Phycobiont von *Catolechia wahlenbergii*. Pl. Syst. Evol. **136**: 63–72.

Watanabe S. 1977. The genus *Chlorella* (Chlorococcales) from Japanese soils (2). J. Jpn. Bot. **52**: 129–136.

花方信孝[a, b]，軽部征夫[b]，千原光雄[b, c]：日本産樹皮付着性緑藻（3）

内房地区（千葉県）の樹木皮より単離した*Chlorella* 2種について分類学的研究を行った．1種は細胞が卵形または楕円形で，他の種は極端な長楕円形であった．前者の葉緑体は，若い細胞では帯状あるいは皿状であるが，成熟すると不定形でしばしば細胞壁から離れ，また，自生胞子の大きさは異なっている．後者の葉緑体の形状は基本的には前者と類似するが，自生胞子のサイズはほぼ等しい．両種ともピレノイド基質は多数のデンプン粒およびピレノグロビュールにかこまれ，また，チラコイドが湾曲して貫通している．上記の形質から，前者を*Ch. trebouxioides*と同定した．一方，後者は*Chlorella*属の特徴を有するが，これらの特徴をもつ既知種がないので新種とし，*Ch. angusto ellipsoidea* Hanagawa & Chiharaの種名を用意した．

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